

WE CLAIM:

1. A microplate for holding a plurality of samples, the microplate comprising:

a frame; and

5 a plurality of sample wells disposed in the frame, at least one sample well having a wall capable of transmitting light, a portion of the wall having an inner surface and an opposing outer surface, wherein the inner and outer surfaces are not parallel.

10 2. The microplate of claim 1, each sample well having a wall capable of

transmitting light, the wall having an inner surface configured to contact a sample held within the sample well and an outer surface capable of transmitting light incident on the outer surface to the inner surface, wherein at least a portion of the inner surface is substantially flat, and wherein at least a portion of the outer surface makes an angle

15 greater than about 42° degrees with respect to the substantially flat portion of the inner surface.

3. The microplate of claim 1, the wall having an index of refraction, wherein the angle is chosen so that light incident on at least a portion of the outer surface along a normal to that portion of the outer surface will be totally internally reflected at the inner surface when the sample well is empty.

5

4. The microplate of claim 1, the wall having an index of refraction, wherein the angle is chosen so that light incident on the outer surface along a normal to the outer surface will be totally internally reflected at the inner surface when the sample well contains water.

10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20

5. The microplate of claim 1, the wall having an index of refraction, wherein the index of refraction is at least about 1.3.

15

6. The microplate of claim 1, the at least one sample well including a side wall and a bottom wall joined to the side wall, wherein the bottom wall is the wall capable of transmitting light.

20

7. The microplate of claim 6, the side wall having an inner surface, wherein the inner surface of the side wall and the inner surface of the bottom wall form a frustum of cone having a cone angle of at least about 8 degrees.

5

8. The microplate of claim 1, the outer surface forming a frustum of cone.

100 90 80 70 60 50 40 30 20 10

9. The microplate of claim 1, the outer surface forming at least a portion of a spheroid, ellipsoid, or paraboloid.

15

10. The microplate of claim 1, wherein the inner surface includes a coating capable of increasing the penetration of the evanescent field into the sample well.

11. The microplate of claim 1, the at least one sample well having an open end through which sample may be added or removed, wherein the wall capable of transmitting light is opposite the open end.

20

12. The microplate of claim 1, wherein the at least one sample well is radially symmetric.

5 13. The microplate of claim 1, wherein the frame is substantially rectangular.

14. The microplate of claim 1, wherein the wall capable of transmitting light includes at least one of the following compositions: plastic, glass, and fused silica.

15. The microplate of claim 1, wherein the wall is formed of a composition that substantially maintains the polarization of incident light.

15

16. The microplate of claim 1, wherein the frame is configured to function as a wave guide, so that multiple internal reflections may be used to create simultaneous evanescent fields adjacent the inner surfaces of at least two sample wells.

17. The microplate of claim 1, wherein the microplate includes at least about 384 sample wells.

5        18. The microplate of claim 1, wherein each sample well holds no more than about 55 microliters.

10      19. The microplate of claim 18, wherein each sample well holds no more than about 5 microliters.

15      20. The microplate of claim 1, wherein the thickness of the wall capable of transmitting light is not uniform.

21. A method for detecting luminescence emitted by a sample, the method comprising:

providing a microplate having a plurality of sample wells, at least one sample well having a wall capable of transmitting light, the wall having an inner surface configured to  
5 contact a sample held within the sample well and an opposing outer surface that is not parallel to the inner surface;

directing excitation light through the outer surface so that it impinges on the inner surface at an angle sufficient for total internal reflection, so that an evanescent field is created in the sample well; and

10 detecting luminescence emitted by a sample within the sample well in response to excitation by the evanescent field.

22. The method of claim 21, wherein each sample well has a wall capable of  
15 transmitting light, the wall having an inner surface configured to contact a sample held within the sample well and an opposing outer surface.

23. The method of claim 22 further comprising:

directing excitation light through the outer surface of a second sample well so that  
the excitation light impinges on the inner surface of the second sample well at an angle  
sufficient for total internal reflection, so that an evanescent field is created in the second  
5 sample well; and

detecting luminescence emitted by a sample within the second sample well in  
response to the evanescent field.

10 24. The method of claim 23 further comprising correlating a difference in  
luminescence detected from the first and second sample wells with a difference in a  
property of the samples in the first and second sample wells.

15 25. The method of claim 21, the at least one sample well including a side wall

and a bottom wall joined to the side wall, wherein the bottom wall is the wall capable of  
transmitting light.

26. The method of claim 25, wherein the side wall and bottom wall form a frustum of cone having a cone angle of at least about 8 degrees.

5        27. The method of claim 21, wherein the directing step includes orienting the direction of excitation light substantially normal to the outer surface.

10      28. The method of claim 21, wherein the wall capable of transmitting light includes at least one of the following compositions: plastic, glass, and fused silica.

15      29. The method of claim 21, wherein the inner surface includes a coating capable of increasing the penetration of the evanescent field into the sample well.

30. The method of claim 21, wherein the step of directing excitation light through the outer surface includes orienting the excitation light so that it is transmitted through the outer surface substantially without changing direction.

31. The method of claim 21, wherein the exciting light is substantially collimated.

5       32. The method of claim 21, wherein the step of detecting luminescence emitted by the sample includes positioning a detector so that the angle between the incident excitation light and detected emission light is substantially different than 0, 90, and 180 degrees.

10      33. The method of claim 21, wherein the step of detecting luminescence emitted by the sample includes positioning a detector so that it is not in the path of the excitation light or a principal reflection of the excitation light.

15      34. The method of claim 21, wherein the luminescence emitted by the sample is used in a luminescence intensity assay, a luminescence polarization assay, or a luminescence resonance energy transfer assay.

35. The method of claim 21 further comprising correlating the luminescence emitted by the sample with binding between a first and second binding partner, at least the second binding partner being associated with the sample.

5

36. The method of claim 35, wherein the first binding partner is the inner surface.

10  
15  
20  
25  
30  
35  
40  
45  
50  
55  
60  
65  
70  
75  
80  
85  
90  
95

37. The method of claim 35, wherein the first binding partner is bound to the inner surface.

15 38. The method of claim 35, wherein the luminescence emitted by the sample is enhanced by the binding between the first and second binding partners.

39. The method of claim 35, wherein the luminescence emitted by the sample is diminished by binding between the first and second binding partners.

20

40. The method of claim 35, wherein at least one of the first and second binding partners is a protein or a nucleic acid.

5       41. The method of claim 21, the excitation light being directed through the outer surface at a first angle to form a first evanescent field characterized by a first penetration depth, further comprising:

            directing the excitation light through the outer surface at a second angle to form a second evanescent field characterized by a second penetration depth; and

10       detecting luminescence emitted by the sample in response to excitation by the second evanescent field.

15       42. The method of claim 41, further comprising correlating the difference in luminescence detected from the first and second evanescent fields with a property of the sample.

43. The method of claim 21, wherein the step of directing excitation light through the outer surface includes orienting the excitation light so that it impinges on the outer surface substantially along a normal to outer surface.

5

44. The method of claim 21, wherein the step of directing excitation light through the outer surface includes orienting the excitation light so that it impinges on the outer surface substantially off a normal to reduce back reflections.

10

45. The method of claim 21, the sample including bulk solution and the solution near the inner surface, further comprising discriminating between

15

46. The method of claim 21, wherein the steps of directing and detecting include illuminating, waiting, and detecting, so that short lifetime luminescence substantially decays before detection.

47. A system for detecting luminescence emitted by a sample, the system comprising:

a sample holder having a frame and a plurality of sample wells disposed in the frame, each sample well configured to hold a fluid sample; and

5 an optical device having an examination site, a light source positioned to deliver light to the examination site, and a detector positioned to receive light transmitted from the examination site;

wherein the sample holder and optical device are configured so that when the sample holder is positioned in the examination site, the optical device is capable of exciting and detecting luminescence substantially exclusively from a sensed volume adjacent an inner surface of at least one sample well in the sample holder.

48. A system for detecting luminescence emitted by a sample, the system comprising:

a sample holder having a frame and a plurality of sample wells disposed in the frame, each sample well configured to hold a fluid sample; and

5 an optical device having an examination site, a light source positioned to deliver excitation light to the examination site, and a detector positioned to receive emission light transmitted from the examination site;

10 wherein the sample holder and optical device are configured so that when the sample holder is positioned in the examination site, the optical device is capable of exciting and detecting luminescence from the sample substantially without carrying light energy into a sample well.

15 49. A method for detecting luminescence emitted by a sample, the method comprising:

directing light substantially normal to an exterior surface of a microplate well sample container;

totally internally reflecting the light from an internal surface of the container; and

20 detecting luminescence emitted by a sample within the sample well in response to excitation by the evanescent field.

50. A microplate for holding a plurality of samples, the microplate comprising:  
a frame; and  
a plurality of sample wells disposed in the frame, at least one sample well having a  
wall capable of transmitting light, the wall having a nonuniform thickness that provides  
5 an optimal angle of incidence of incoming light through an outer surface of the well and  
total internal reflection at an opposing internal interface in the well.